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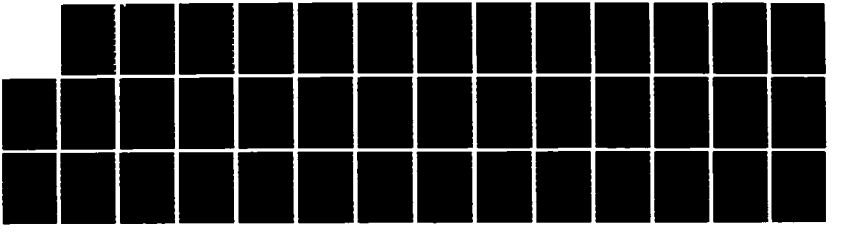

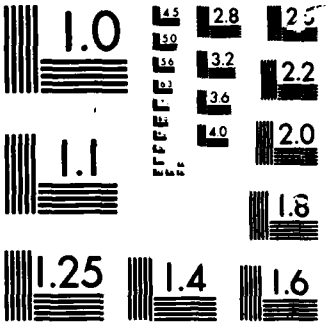

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Research supported by this Contract was conducted in two phases. The first was entitled "The Use of Double Translocation Heterozygotes to Control Populations of the German Cockroach." The second focused on behavioral research and was entitled "The Use of Genetic Mechanisms and Behavioral Characteristics to Control Natural Populations of the German Cockroach."

Research under Phase I was directed towards developing and testing a possible mechanism for genetic control, specifically, double translocation heterozygotes. An initial laboratory experiment showed that releasing males carrying a single 2-chromosome translocation retarded but did not suppress population growth. Double translocations impart a higher genetic load. Their potential for suppressing and possibly eliminating infestations is substantially increased by an egg case sterility effect, "embryonic trapping." The sterility effect occurs when lethality exceeds 50%. Statistical analyses of the lethality-sterility relationship showed that the frequency of sterile oothecae increases sharply with small elevations in lethality in the range of 50-80%. Above that, complete sterility is expected. Sterility from

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terile translocations easily falls within this range.

The first requirement for development of phenotypically identifiable double-translocation arrying males was to find mutant markers closely linked to single translocations. Subsequent enetic manipulations were used to develop three crossing systems. Two perpetuated each single ranslocation. The third was intercrosses between the translocations. Mutant markers were tilized in such a way that double-translocation carrying individuals were distinguishable from ther genotypes among the progeny by their normal phenotype. Three double translocations were eveloped and analyzed cytogenetically. In the first two, combined lethality-sterility effects educed mean hatch per ootheca from a normal of 40 nymphs to 2.5 $\pm$ 0.7 and 0.7 $\pm$ 0.2, espectively. Laboratory population experiments with each double showed growth was suppressed, ut suppression was obtained more rapidly with the double that had the greater hatch reduction. he third double was developed late in the contract. A sex difference in fecundity of an nusual 3-chromosome progressive interchange suggested it might be possible to obtain non-terile matings of one sex (female) to one of the single 2-chromosome locations. If so, the ouble males among the progeny carry a mean lethality of > 80% and presumably their matings to ild-type females would be completely sterile. Both possibilities were realized. It was ecided to use this unique sterile male in a pilot field test of genetic control.

Cytogenetic analyses added to the understanding of chromosome disjunction in translocation eterozygotes. For the first time in an animal organism, the existence of alternate-2 disjunc-ion was demonstrated. Surprisingly regular ratios of four cell types were found to underlie requencies of alternate and adjacent disjunction that characterize particular translocations.

Several experiments were directed toward obtaining information requisite to planning a ield trial. They included studies on male competitiveness, insemination frequency, and ge-class structure and density of populations on two ships where the field trial was to be onducted. Translocation-carrying males were slightly more competitive than males collected s nymphs from one of the ships. Monogamy predominated, although re-mating sometimes occurred. removal method of population estimation provided estimates of immatures. Numbers of males to e released were predicted on numbers of females expected to mature within intervals between each elease.

The rationale for the field trial was that sterile males could be used to suppress growth nd possibly eliminate residual spot infestations that remain after an insecticide treatment. he experiment, on an LST, could not be comple:ed, due to operational requirements for the LST. he other, on an ocean-going tug, did not suppress population growth. Growth of groups that nvaded new and undetected harborages following the preliminary insecticide treatment was pparently uncontrolled. Sterile males joined groups that were near the sites of release and hey competed well against wild-type males. Analysis of egg-case sterility was a useful tool in stimating the frequency of sterile matings.

Contributions from the above research included an enhanced understanding of chromosome ehavior in translocation heterozygotes, discovery of a unique sterility mechanism, development f a double translocation-carrying male that causes complete sterility in matings to wild-type males, and utilization of the sterile male in the first field trial of a genetic mechanism as rt of a cockroach control program. The latter experiment revealed a need to improve the echnique of field application through a more in-depth knowledge of populations behavior; the enetic mechanism itself was successful from the point-of-view of its introduction into the ipboard population.

Research during Phase II was a blend of laboratory and shipboard experiments that increased r understanding of population behavior and provided information that can be used to enhance rrent control procedures and to design programs that include new control techniques. Contribu-ons of particular value were from shipboard experiments where we were given an opportunity to tablish populations on an inactive ship. Free populations are generally difficult to study cause they occur where they are subject to uncontrolled disturbance by people. Estimates of pulation growth ~~from the first~~ shipboard experiment indicated that free populations developing der essentially optimal conditions would show a 20 to 26-fold increase in a 3-month period. apping data showed that: peaks of highest density in a thoroughly-trapped area indicated the cation of spot infestations; catch close to localized aggregations gave reasonably good dications of relative density; traps between spot infestations could not be used for density

19. (continued)

estimations; and that age class frequency in traps was unlike that in nearby infestations. Age class structure of rapidly-growing groups differed from that of groups where harborage availability limited growth. Evidence that middle to late instars are the primary migrants between groups was supported by laboratory experiments. Laboratory experiments also showed that group response to increased female density depended on female reproductive state. The results left little doubt that females play a leading role in regulating population behavior. Subsequent research on pheromones showed that other age/sex classes alter their behavior in response to differences in secretions from females in different reproductive states.

The last three shipboard experiments were on the effects of propoxur treatment on populations established in known locations. In each case, immediate dispersal was followed by movement of the majority of the remaining population back to or close to its original location. Nevertheless, small spot infestations were established and remained in previously uninhabited locations, some at a considerable distance from the original site. In two experiments the populations were equally divided between resistant and susceptible (orange-body) cockroaches. Kill of susceptible cockroaches, especially the adults, was higher than that of cockroaches with a 6 to 7-fold resistance to propoxur. Complementary laboratory studies revealed a strain difference in dispersal behavior. Resistant cockroaches were less responsive to propoxur vapors. This finding was supported by additional experiments on rates of movement and antennal and tarsal cleaning.

The relationship between food and water consumption and female reproductive state was also studied. Peaks of water and food consumption occur during the egg maturation period, but terminate abruptly with egg case formation. The effects of water and food deprivation on reproduction and mortality vary with the part of the reproductive cycle during which these resources are not available. The results of the experiments suggest females are well adapted to surviving temporary food shortage. The results have implications for the use of sanitation for cockroach control.

Studies on aggregation pheromone and a less well known dispersal pheromone revealed differences in response with age/sex class. In the aggregation experiments, variation occurred in clustering behavior, as well as in relative attraction of the pheromone. Maximum secretion of both phermones was by 7-10 day-old non-gravid females. Subjecting males and large nymphs to levels of crowding equal to that which causes females to secrete sufficient dispersal pheromone to elicit a strong response gave negative results in respect to repellency.

The results of the research on cockroach behavior indicate that population response to chemical stimuli, both natural (pheromonal) and artificial (insecticidal), will not be the same for all its members. Females play a leading role in regulating population behavior. The existence of a strain difference in behavior of one resistant strain suggests that the development of resistance in other field populations may also be accompanied by alterations in behavior.

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FINAL REPORT

THE USE OF DOUBLE TRANSLOCATION HETEROZYGOTES TO CONTROL POPULATIONS OF THE GERMAN COCKROACH

and

THE USE OF GENETIC MECHANISMS AND BEHAVIORAL CHARACTERISTICS TO CONTROL NATURAL POPULATIONS OF THE GERMAN COCKROACH

by

Mary H. Ross, Ph.D., Principal Investigator, and Donald G. Cochran, Ph.D., Co-principal Investigator

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June 1986

ONR-supported research on the German cockroach, Blattella germanica (L.), has gone through two main phases. One was concerned with the possibility of using a genetic mechanism for cockroach control. This research covered a span of 6 years, during which the Contract was entitled "The Use of Double Translocation Heterozygotes to Control Populations of the German Cockroach" (1975-1980). The culmination of this research was the first field test of a genetic control mechanism in a cockroach (Ross et al. 1981). The results brought to the fore a dearth of knowledge on the behavior of natural populations. Therefore, continued research was focused on behavioral studies. During these years (1980-1986), the contract was entitled "The Use of Genetic Mechanisms and Behavioral Characteristics to Control Natural Populations of the German Cockroach".

The paragraphs below present an overview of the research according to each of the phases noted above. Key reprints from the earlier work are appended. All reprints from more recent research are also included. A complete publication list may be found on p. 31.

PHASE I. RESEARCH ON GENETIC CONTROL.

The best known genetic control technique is the sterile male, where sterility has been induced by ionizing radiation. However, a number of other genetic mechanisms

are known that have potential for population suppression and/or elimination. One is chromosome translocations. This type of chromosome mutation has zygotic lethal effects due to gametes that carry unbalanced chromosome complements. At the time this Contract was initiated, we had 20 translocations in stock. Completion of cytogenetic analyses on three (Ross and Cochran 1975, Cochran and Ross 1977a) brought the number of those with known lethal effects and chromosome identification up to ten. Most showed good male competitiveness. Furthermore, each translocation (translocation heterozygote) was established in a backcross system with a closely linked mutant marker. This meant that translocation-carrying individuals could be identified visually by phenotype.

The reproductive potential of the German cockroach is very high. Single 2-chromosome translocations, at best, do not reduce hatch by more than 50%. A laboratory study on release of single translocations conducted early in the Contract showed that population growth was substantially retarded, but was not suppressed (Ross 1975). Therefore, subsequent research was concentrated on an unusual three-chromosome translocation, on combining translocations (double translocation heterozygotes), and on other studies relevant to developing and testing genetic mechanisms that were potentially capable of suppressing growth of field populations.

a. Male competitiveness (Ross 1978). The factor that has played the major role in a lack of success in field trials of genetic mechanisms in other insects is that the introduced males (those carrying the deleterious trait) did not compete well with wild type. A laboratory test on male competitiveness of B. germanica showed (1) a tendency for males from a laboratory-translocation stock to outcompete those collected as nymphs from a Norfolk-based ship and (2) retention of competitiveness by unmated males throughout the 1st mo of adulthood, with a slight peak in aggressiveness at ca 2 wk. The act of mating was followed by a reduction in competitiveness. Therefore, it would be advantageous to hold unmated males to be released for genetic control for about 2 wk post-ecdysis. The experiment was also very promising from the point-of-view of the ability of laboratory-reared, translocation-carrying males to compete with wild type.

b. Insemination frequency (Cochran 1979). A question of fundamental importance to the biology of the species and to the use of a genetic control mechanism is the number of times a female is successfully inseminated. Experiments utilizing a genetic marker indicated that German cockroaches are generally monogamous, although monogamy is not complete.

c. Formal genetics (Ross 1975). The inheritance patterns and linkage relationships of three new mutants were described. Two were eye colors and the third an alteration in body form. Each was identifiable in nymphal stages.

Therefore, they increased the pool of genetic markers that might be used to identify translocation-carrying males before adulthood.

d. Basic cytogenetic studies on single translocation heterozygotes (Ross and Cochran 1975; Cochran and Ross 1977a, 1977b). Three 2-chromosome translocations, each involving chromosome 12, i.e., T(3;12)/3;12, T(7;12)/7;12, and T(11;12)/11;12, were analyzed cytogenetically and placed in backcross systems to closely-linked genetic markers. T(7;12)/7;12 was characterized by a sex difference in fecundity wherein lethality in outcrosses of females to wild-type males was higher than in the reciprocal cross. A similar difference was found in analyses of an unusual 3-chromosome translocation, except that hatch from both reciprocal crosses was extremely low. This was due in part to a higher lethality, as expected from involvement of three rather than two chromosomes. However, an unusual sterility mechanism that accompanies high lethality played a major role in hatch reduction (see "e" below). In meiotic cells of both the 2-chromosome and the 3-chromosome translocations, the translocation configuration (multivalent) was generally maintained in the form of rings at metaphase I by a terminal chiasma in each arm. This property is common to translocations in B. germanica. However, translocations with random chromosome disjunction (ca. 50% lethality) show a tendency towards more frequent breakup of rings into chains-of-four than in translocations

characterized by directed alternate disjunction (favoring of alternate disjunction results in a higher proportion of gametes carrying balanced chromosome complements and hence in lethalities below 50%).

Peculiar features of translocation heterozygotes in B. germanica made it possible to identify a particular ring configuration as alternate-2 disjunction (Cochran 1976, 1977). The existence of this disjunction type has been postulated on theoretical grounds and demonstrated in a plant organism. Its identification in B. germanica was the first for an animal organism. Another feature of considerable interest was that the four types of disjunction occurred in regular ratios. Furthermore, a favoring of alternate disjunction could be attributed to a single disjunction type, i.e., a higher frequency of alternate-2. The results of the basic research added to the understanding of chromosome disjunction and other cytogenetic properties of translocation heterozygotes.

e. A unique sterility mechanism (Ross and Cochran 1967; Keil and Ross 1977, reprint appended). Hatch reductions in matings between translocation heterozygotes were grossly in excess of those predicted on basis of known lethal effects. An investigation of this phenomenon showed that reduction in the number of viable embryos in an oothecae could reach a point where their combined strength was unable to force open the ootheca at the time of hatch. We named this occurrence "embryonic trapping". It has little effect at lethalities

below 50%. An analysis of the relationship between embryonic lethality and trapping showed that the percent of sterile oothecae increased sharply with relatively small increases in lethality in the range of 50-80%. A modified constrained regression gave a means of predicting expected hatch from lethality caused by high genetic loads. Complete sterility was predicted at 82% lethality.

f. Synthesis and study of double translocation heterozygotes (Ross and Cochran 1977, 1979, 1981). The double translocation heterozygotes developed and analyzed in the foregoing studies included one that combined two independent 2-chromosome translocations, a 3-chromosome double that combined two translocations that had one chromosome in common, and combination of a 2-chromosome translocation with a progressive interchange involving three chromosomes, respectively.

The first step in the synthesis of phenotypically identifiable double translocation carrying cockroaches was to develop a double mutant stock, homozygous for each of the mutants used to identify the single translocations. Next, each single translocation was established in a backcross system to the double mutant stock. For example, suppose that two translocations, T^1 and T^2, were identified by orange-body (or) and ruby-eye (ru), respectively. In backcrosses to or, ru, T^1 would be normal for or but phenotypically ru; T_2 would be normal for ru but phenotypically or. In intercrosses between T^1 and T^2, the

double would be phenotypically wild type. In short, sythesis of each double required three crossing systems, two consisting of backcrosses of each translocation to a double mutant stock and the third consisting of crosses between the single translocations.

Each of the three types of double translocation-carrying males noted above were fully competitive with wild type. Therefore, choice of one for a pilot field trial was largely on the extent of hatch reduction in matings to wild-type females. The combined effects of embryonic lethality and trapping in the 4-chromosome double reduced the mean hatch from a normal of ca. 40 nymphs/ootheca to 2.5 $\pm$ 0.7. In the 3-chromosome double, hatch was reduced to 0.7 $\pm$ 0.2. Productivity in intercrosses requisite to synthesizing the double males was closely similar. The results from the 3-chromosome double were sufficiently promising to warrent a laboratory study on population suppression (see below). The third double, combining a 2-chromosome and 3-chromosome single translocation, was the last to be developed. A sex difference in fecundity favoring females of the 3-chromosome single raised the possibility that crosses to a 2-chromosome translocation might be productive, in spite of high lethality from involvement of five chromosomes. If so, it might be possible to obtain double males that cause complete sterility from embryonic trapping, since they would carry a lethality of slightly over 80%. Both possibilities were

realized. As a result, a male imparting complete oothecal sterility was available for field testing.

g. Laboratory population experiments (Ross 1975, 1977, 1980). The first study used repeated releases of a single translocation heterozygote. Population growth was retarded, but not suppressed. In studies with a 4-chromosome double, it was necessary to continue releases into the F_1 and F_2 generations in order to build up a sufficient genetic load to cause the population to decline. Better results were obtained with the 3-chromosome double, as expected from analyses of its basic properties noted above. The experimental population declined following release of double males into one generation of progeny.

h. Studies on free populations (Ross and Wright 1977, Ross 1981, Keil 1981). The first detailed analysis of the age class structure of natural populations was on populations collected by C. G. Wright from single family dwellings in North Carolina. House to house variations occurred, but were minimal compared to differences from laboratory populations. A possible explanation was a difference in primary regulating factors. Containment of laboratory populations limits population growth. Here crowding could be responsible for high nymphal mortality. In contrast, evidence of inhibited productivity in field populations could be explained by known effects of scarcity of water and food on mating and oothecal formation.

An important component of field trials is the preliminary assessment of population size and location of spot infestations and post-release monitoring of the population. A small experiment on trapping with mason jars vs roatel traps (Ross 1977) showed that the roatel caught about four times as many small nymphs as in the jars, but otherwise results were similar. Roatel traps were the better choice for the field trial since the first effect of successful matings of sterile males would be a decrease in hatch.

A graduate student who received partial support from the ONR Contract, C. B. Keil (now Asst. Prof. Entomol. at Univ. Deleware), conducted research on population structure and density on two ships selected for field testing of the 5-chromosome double translocation males. Arrangements for the study were made through the cooperation of Environmental and Preventive Medicine Unit 2 at the Norfolk Naval Base. A removal method of population estimation was applied primarily because information could be obtained on the density and age class distribution of immatures. The rationale for the upcoming field trial was that sterile males be released to suppress population regrowth after a standard insecticide treatment. Therefore, the estimates were on both pre- and post-treatment populations. Post-release estimates provided the basis for deciding on numbers of sterile males to be released. Also, the location of spot infestations was used as a guide to sites of release.

i. Genetic control experiment (Ross et al. 1981). The rationale for the genetic control experiment was that sterile males (double translocation heterozygotes) could be used to suppress growth from and possibly eliminate small residual groups that remain following standard insecticide treatments. They were released on two ships, with the majority on a LST (USS Boulder) and much smaller releases on an ocean going tug (USS Papago). Males were released at sites preselected in the preliminary study by Keil (spring of 1980). The experiment on the USS Boulder was not completed due to circumstances beyond our control involving Navy operations.

Three releases of sterile males were made at monthly intervals for three months on the USS Papago. The experiment was terminated at 4-1/2 months. The results showed that released ♂♂ joined groups near release sites and that they competed well against wild type ♂♂. Apparently neither they nor ♀♀ with which they mated moved far from these sites. Sterility effects differed in respect to specific sites and general areas. Population growth was retarded markedly in the galley, the area of heaviest initial infestation. Terminal infestation was heaviest in the mess deck, although the highest sterility among ♀♀ occurred at/near the mess-deck release site (only one mess deck-infested site was found prior to initiation of the experiment). It is suggested that a slight increase in number released would have suppressed/eliminated groups

inhabiting galley harborages, but that site selection was the major problem in the mess deck. Analyses of nymphal age classes and mating types among ♀♀ led to a hypothesis that insecticide-induced dispersal of nymphs resulted in the infestation of many new mess-deck harborages. Population growth was unchecked at these sites because they were too far removed from the sterile male release sites.

Both the preliminary population studies of Keil and the genetic control experiment were pioneer efforts for the German cockroach. The removal technique of population estimation served as a reasonably reliable guide in judging the relative size of releases needed at known harborage sites. The major factor in a lack of overall population suppression was apparently the unrestricted growth of groups that invaded new and undetected harborages as a result of insecticide-induced dispersal. Within localized aggregations, the sterile males joined and competed well against wild-type males. Analyses of egg cases (sterile vs non-sterile) proved to be an uniquely useful tool in estimating the frequency of sterile matings and also as an indication of the time and place where sterile matings occurred.

This first experiment in the use of a sterile male technique for the German cockroach pointed to several aspects of wild-type behavior and population dynamics that need further exploration. The availability of such information would be useful both from the point-of-view of

more effective application of conventional control methods and the development of integrated programs for cockroach control. Increased population density on the experimental ship reflected this situation rather than any defect in the genetic tool itself.

The ease with which sterile males were introduced into the populations and their tendency to remain at or close to sites of release augered well for the use of genetic markers in studies of dispersal, migration, hatch, nymphal development, and survival under shipboard conditions. The major thrust of our effort during the continuation of this Contract was on these and related types of study. Nevertheless, the utilization of sterile males in future experiments in genetic control remained a viable option as long as the translocation stocks continued to be available. With termination of ONR support, most translocation stocks will be discontinued. They are not available elsewhere.

PHASE II. POPULATION BEHAVIOR AND RELATED STUDIES

Research conducted during this phase of the Contract was a blend of laboratory and shipboard experiments that added to our basic understanding of cockroach behavior and made available information of value to control efforts. Experiments included studies on growth and behavior of undisturbed populations and on response to naturally-occurring stimuli (pheromones) and to insecticides. A uniquely valuable component of this work was the opportunity

to study free populations that we established on an inactive ship, the USS Gilmore. Continued cooperation from Navy Environmental and Preventive Medicine Unit 2, especially of our liason officer, LCDR Donald McCroddon, and of the Portsmouth Inactive Ship Facility made these experiments possible. A distinctive feature of all experiments was that we differentiated among responses according to female reproductive state, adult sex, nymphal stage, and also strain. This has rarely been done in other studies on cockroaches. The results revealed that dispersal behavior differs with age/sex class. They included the first evidence of a strain difference in behavior of B. germanica. Also, the combined findings from several experiments supported an hypothesis that adult females play a major role in governing population behavior.

This portion of the Contract (1980-86) included support for a graduate student, Brian L. Bret. Dr. Bret is now with Western Termite & Pest Control Company. The references included in the paragraphs below document Dr. Bret's contributions to ONR-supported research.

a. Group and population behavior. This research included laboratory (Bret and Ross 1983, 1984, 1985a) and shipboard (Ross et al. 1985) experiments.

The initial laboratory study was on the spacial distribution of mixed-age groups within four adjoining shelters. Female density and reproductive state were varied; nymphal components of the groups were constant.

Differences with age/sex class included stronger aggregation of small nymphs than middle instars and a general lack of significant aggregation among males. The most unexpected finding was that changes in the intensity of aggregation with increased density of gravid (egg case-bearing) females were opposite to those found with non-gravid females. Aggregation increased with increased density of gravid females, with a more intense aggregation if females carried immature rather than mature oothecae. It decreased with increased density of non-gravid females. The results of the experiments were attributed to differences in response to varying amounts and types of chemical stimuli secreted by adult females, specifically, the aggregation pheromone and a dispersant pheromone females secrete when crowded (see "c" below). The most likely explanation of the difference in response to increased density of gravid and non-gravid females is that the level of stress (crowding) necessary to trigger dispersant secretion by non-gravid females is lower than that for gravid females.

In a second laboratory study on mixed age groups dispersal behavior was investigated. The adult females were either gravid at the start of the experiment or formed oothecae during the week-long experiment. Dispersal decreased with increased female density. This behavior, and increased aggregation within shelters with similar increases of density, can almost certainly be attributed to increased amounts of aggregation pheromone due to increased numbers of

adult females. Among individual age/sex classes, early instars were unique in that dispersal remained low at all female densities. Subsequent work on aggregation pheromone (see d) showed they have a particularly strong response to aggregation pheromone.

The above experiments revealed a new facet of German cockroach behavior, as noted in an article in Pest Control Technology (Bret and Ross 1983). Apparently adult females play a leading role in governing population behavior. They are capable of varying their secretion of chemical stimuli (pheromones) with changes in reproductive state and in density.

In the shipboard experiment, groups were established in known locations throughout the galley and adjacent rooms on the USS Gilmore. Population growth and movement were monitored by traps throughout a 4-month period. Much of the population was in cartons left at food-water stations. Collection of the cartons and of cockroaches killed in cleanout treatments of specific areas made possible a reasonably good comparison of trapping data to density. This information is not generally available in the literature. Catch in traps immediately adjacent to spot infestations proved to be good indicators of relative densities of cockroaches in the nearby group and the immediate vicinity. This was not true of traps between infested harborages. In short, when an area is thoroughly trapped, peaks of highest density can reveal locations of

infested harborages. Traps near such harborages can then be used to gain an estimate of relative densities and growth of spot infestations. However, trap catch does not give a reliable indication of age class structure, even when near an infested harborage. Small nymphs are almost certain to be under-represented; the proportion of females caught will vary with reproductive state. In our traps, adult females were over-represented. This can probably be explained by high food-water requirements prior to egg case formation (see b). The population was growing rapidly, and consequently the numbers of newly-matured females (non-egg case bearing) increased continually. Total trap catch throughout the experiment provided the first measure of growth of a free population under essentially optimal conditions. It was concluded that such populations would increase at least 20X in 3 months and possibly as much as 26X. This information can be used to estimate whether and to what extent limiting factors inhibit growth of pest infestations.

Among individual groups, some grew rapidly and others showed little to no growth. Underlying changes in age class distribution distinguished these situations. The results could be explained if small groups were limited by harborage, that group response to this limitation was dispersal of middle to late instars, and that much of the movement was from the small to large groups. Catch in traps most likely to sample migrants indicated middle to late

instars were the primary migrants. Supporting data were found in the dispersal study (Bret and Ross 1985a) and a possible explanation in research on aggregation pheromone (see c). Other findings related to age class distribution were that catch of high proportions of early instars outside an infested harborage was an indication of overcrowding and that migration caused by removal of water and food did not appear to vary with age class. For more information on this experiment, please see the appended reprint (Ross et al. 1981).

One facet of the above shipboard study is unpublished. Hopefully time and funds will be found to rectify this situation. Black-body females carrying first egg cases were released at a site where one of the wild-type groups was established. First instars from three successive oothecae hatched at about monthly intervals. Their distribution indicated the females stayed at or close to the site of their release. Also, the newly-hatched nymphs showed little movement. In contrast, many middle instars moved into new locations. Each group of middle instars from the three sets of oothecae moved farther than its predecessor. One possibility is that they moved beyond any wild-type groups that had reached the limitations of their particular harborage. The latter became more common towards the end of the experiment. A possible implication for genetic control is that it might be difficult to introduce sterile males

into populations that are at the carrying capacity for their particular environments.

b. Food and water consumption by adult females (Cochran 1983, Durbin and Cochran 1985). The initial study examined the relationship of water and food consumption to the female reproductive cycle. Peaks of feeding and drinking occur during the egg maturation period, but are abruptly terminated at the appearance of each egg case. Obviously this impacts on trapping data. The experiment also revealed differences between the first and subsequent reproductive cycles. It was hypothesized that the presence of an ootheca in the genital chamber is an inhibitory stimulus of feeding and drinking and probably corpora allata function as well. Once that stimulus is removed, the females are free to drink and feed and begin a new cycle. If water and food are available, they consume them and thereby trigger the hormonal mechanism necessary for egg development. If not available, the reproductive process may be held in abeyance as long as the female survives. The results suggested the females would be particularly vulnerable to food and water deprivation during the period when they are forming an egg case. This was investigated in a follow-up study. Females survived much longer without food than they did without water. Mortality varied greatly according to the part of the reproductive cycle during which food was not available. For example, considerable mortality occurred in females deprived for 12 days following maturation whereas all those

with first oothecae survived without food for the entire incubation period (> 20 da). Both food and water deprivations caused delays in the reproductive cycle and decreased oothecal hatch. Following food deprivation, females tended to compensate by eating well. They appear to be well-adapted to surviving temporary food shortages. By having a reproductive cycle where certain periods are more important than others in terms of nutritional requirements, the females may be able to reproduce more efficiently.

c. Insecticide-induced behavior, Experiments were conducted in the laboratory (Bret and Ross 1985b, 1986) and on shipboard (Ross and Bret 1986). The detailed reports on these studies are appended either as reprints or manuscripts currently in press.

A laboratory experiment on insecticide-induced dispersal showed that propoxur vapors drove cockroaches out of an aquarium with essentially optimal conditions to one where water and food were lacking. The repellency of the formulation (Octogon Roach Spray) was immediate (occurring within the first 15 min) and persistent for at least 24 h. The behavior of two strains, a laboratory susceptible strain (VPI) and a propoxur-resistant field strain (Bowling Park), was investigated. Dispersal in control experiments was closely similar, but the strains responded differently to the propoxur vapors. The difference was due to a significantly faster dispersal of VPI than Bowling Park strain adults. Since the VPI strain has never been exposed

to insecticides, we inferred that the development of physiological resistance in the BP strain was accompanied by a decreased dispersal response of adults. It is unclear why nymphs of the BP strain responded similarly to VPI strain nymphs. In any case, this study was the first to show a strain difference in behavior in B. germanica. Variation in behavioral responses to insecticides among field strains has implications for the efficacy of control measures.

The above study on insecticide-induced behavioral responses of susceptible (VPI) and resistant (BP) cockroaches was continued in experiments on adult males. Males were used because the dispersal experiment showed that the greatest inter-strain difference was in males, although adult females of the two strains also differed significantly. Three experiments were conducted. One investigated excitatory behavior as seen in movement within an enclosed area. The others were on antennal and tarsal grooming behavior. They were measured as a response to the irritant characteristics of the propoxur formulation. In both strains, the most immediate response to propoxur vapors was increased movement. This was followed by increased antennal grooming and last by increased tarsal cleaning. Antennal grooming decreased after 10 min. The most likely explanation was intoxication. That is, the cockroaches suffered a loss of motor coordination that resulted in unsuccessful attempts to clean the antennae. The more delayed response of tarsal grooming often followed

unsuccessful attempts to clean the antennae. In the control experiments, the BP strain males were more active than the VPI males. This was not the case in the dispersal experiment. Cockroaches in the dispersal experiment were provided with water, food, and shelter, whereas those in the movement and grooming experiments did not have these resources. Possibly the absence of resources stimulated a faster response by the BP (field) strain males than by the laboratory-reared VPI males. Beacuse of the difference in the control data, the comparison of responses to propoxur vapors was considered in light of the amount of change between the control and experimental results for each strain. The VPI males consistently showed the greater response relative to the controls. This supports the difference seen in the dispersal experiment.

That present-day populations differ in type and level of insecticide resistance is well documented, but until the above study behavioral differences had not been examined. Behavioral characteristics of resistant populations and of the impact of behavioral changes on control strategies is an important area for future investigation.

Shipboard experiments on insecticide-induced behavior were conducted during the summers of 1982 and 1983. The opportunity to continue research on the same ship used earlier (USS Gilmore) was a considerable advantage to the planning and execution of the experiment. The first experiment (expt. 1) and second experiment (expt. 2) used

the same trap sites. Expt. 3 was in a different part of the ship because it was necessary to conduct it concurrently with Expt. 2. The original plan was to conduct all experiments in the same place, but unfortunately the first one in 1983 was disturbed by rats. In Expt. 1 a propoxur-resistant field strain was used, except that adult males were from a black-body strain (Bl). The latter were distinguished from wild-type males that matured within the population. One advantage here was that Bl males could be used to compare relative effects of two "cleanout" treatments (very thorough and heavy) at the end of the experiment. One was against a large population; the second, against a small residual population. The results support an hypothesis that insecticides are more effective against large than small populations. The population was established at a site known to favor development of a large group. Following a relatively light treatment with propoxur, traps were used to follow distribution for an 8-wk period. Treatment was followed by immediate dispersal. The majority moved into nearby untreated areas, but some dispersal into relatively distant locations also occurred. By 1-wk post-treatment, the majority of cockroaches in nearby areas had moved back to or close to their original location. However, a few new spot infestations were established and remained throughout the duration of the experiment. This was also true of cockroaches that moved

into more distant areas, where they formed new spot infestations close to water/food stations.

In the 2nd and 3rd shipboard experiments, the populations were divided equally between propoxur-resistant and orange-body susceptible cockroaches. The experiments were terminated at 2 wk post-treatment because the initial experiment indicated insecticide-induced changes in distribution would have stabliized with this period. The pattern seen in Expt. 1 was repeated. That is, immediate dispersal into nearby areas was followed by return of most cockroaches to their original locations, but spot infestations were established in new locations. No difference in pre- or post-treatment distribution of resistant and susceptible cockroaches was apparent. However, the possibility that susceptible adults that survived treatment dispersed more quickly than resistant adults, as expected from the laboratory study, could not be ruled out since laboratory dispersal was within 2 h and the shipboard assessment of immediate dispersal was at 24 h after treatment. Estimates of initial kill, post-treatment trap catch, and numbers in final collections all documented higher kill of susceptible than resistant cockroaches. The greatest inter-strain difference was due to higher kill of susceptible than resistant adults, although kill of suceptible nymphs was also higher than in resistant nymphs. Kill in the resistant strain did not appear to vary greatly with age or six class. In the susceptible strain, adult

mortality was higher than in nymphs. At least 50% of the cockroaches released on the ship were accounted for in each of the three experiments.

Very little information is available on the effects of resistance on the efficacy of insecticide treatment of free populations. The experiments noted above indicate that a 7- to 8-fold resistance to propoxur could indeed have such an effect. Considerably more information of this type is needed in order to apply appropriate dosages to pest populations. The experiments also indicated that physiological resistance played a major role in the experimental results. Whether or not behavioral characteristics of the resistant strain made a secondary contribution that enhanced survival is unknown although similarity in posttreatment distribution argues against this possibility. The apparent predominance of physiological compared to behavioral resistance might conceivably evolve because the propoxur resistance mechanism assures a higher survival than possible through the capability of B. germanica to increase its escape from the insecticide. It may be that flying insects have a greater capability for the latter than in a primarily crawling insect like B. germanica.

d. Pheromones (Ross and Tignor 1985, 1986a, 1986b). The first two studies were on a dispersal pheromone and the third on aggregation pheromone.

The pheromone studies utilized a bioassay technique patterned after that of Japanese investigators who were the first to report both the aggregation and dispersal pheromones. Small strips of filter paper were placed at equal distances around the margin of a glass chimney. One was a control paper and others exposed to either gravid or non-gravid females. Distribution of test groups was recorded photographically at 10 min intervals for 4 h after insects were released in the jars. The major differences that distinguish our experiments from those conducted elsewhere are that pheromones were from a known source and response was differentiated as to nymphal stage and adult sex.

The first report on the dispersal pheromone focused on comparing responses of 6 age/sex classes. The papers were conditioned by crowding gravid and non-gravid females. All test classes avoided the conditioned papers, but subtle differences occurred in their response. Most initial movement was from the jar bottom onto the control paper. Non-gravid females moved more slowly than other classes; most rapid movement was by small nymphs. Overall, nymphal response to conditioned papers was similar, regardless of nymphal stage. Greatest repellency was to adult males, and second greatest to gravid females. Four of the classes were more repelled by paper conditioned by non-gravid females than by that exposed to gravid females. Non-gravid females were the only class that responded equally. Males also

differed. More were on non-gravid than gravid female-conditioned paper. Since female sex pheromone is secreted by non-gravid females, we suspect repellency of paper conditioned by non-gravid females was partially countered by attraction of the sex pheromone. The results left little doubt that non-gravid females (7-10 da post ecdysis) secrete more dispersal pheromone that gravid females. Undoubtedly this has some type of adaptive advantage. Perhaps when populations contain large proportions of non-gravid females, crowding stimulates secretion of the pheromone and this, in turn, causes aggregations to spread within a harborage to make room for subsequent oothecal formation and hatch. If density began to exceed the carrying capacity of the harborage, the dispersant could serve to trigger dispersal.

The second report on the dispersant compared response to paper conditioned by crowding late instars and adult males to that conditioned by adult females. As expected, papers conditioned by females were again repellent. No repellency was found in experiments with male and nymphal-conditioned papers. Indeed, that conditioned by late instars was somewhat attractive, suggesting response to aggregation pheromone. Either these classes do not secrete the dispersant or they require a higher level of stress to trigger secretion of sufficient amounts to elicit a response. We conclude that control of population behavior through secretion of this pheromone is a prerogative of the adult females.

The reports on the dispersant represent two of four published to date. In contrast, the aggregation pheromone has been under study for many years. Nevertheless, many aspects of aggregation-induced behavior and of secretion remain to be elucidated. Our study was the first to show a difference in response with age/sex class and also that secretion varies with female reproductive state. An incomplete experiment that was abandoned due to termination of the Contract suggested a difference in the composition of aggregation pheromone secreted by males and females. In the bioassay experiment with females, papers were conditioned by exposure to either 10 gravid or 10 non-gravid females for three days. As in the dispersal study, it appeared that 7-10 day-old non-gravid females secrete more of the pheromone than gravid females. This period corresponds to the culmination of a time of high food and water intake prior to egg case formation, as noted earlier (b). All test classes (early, middle, and late instars, adult males, gravid females, and non-gravid females) were strongly attracted to the conditioned papers, yet significant differences occurred in their response. A lesser attraction to middle and late instars is a possible explanation of their greater away-from-harborage movement in laboratory and shipboard studies (a). As in the study on the dispersal pheromone, male response to paper conditioned by non-gravid females suggested the presence of female sex pheromone. The underlying causes of differences in response of the test

classes were clearly complex. They included variation in the amount or compositon of pheromone(s) on gravid and non-gravid female conditioned paper, the sensitivity of test classes, and the degree of social interattraction typical of each class. A very strong tendency to form a single major cluster on one or the other of the conditioned papers indicated a high social interattraction among early instars. This behavior undoubtedly played a role in laboratory dispersal experiments where most early instars remained in a shelter while other members of the groups showed varying tendencies to disperse to a different location.

The pheromone research has added to the understanding of behaviors observed in laboratory and shipboard population studies. It is clear that all members of a population will not respond equally to chemical stimuli, either natural (pheromones) or artificial (insecticides).

CONCLUDING REMARKS

The overall results of ONR-supported research have contributed to the understanding of the genetics, biology, and behavior of the German cockroach. In many instances, the knowledge can be put to use to improve present control strategies and to devise future control programs that include non-insecticidal types of control.

Highlights of Phase I were the discovery and development of a unique type of sterile male and its successful introduction into a shipboard population. The

distribution of females that had mated with the sterile males indicated that neither the males nor their mates strayed far from groups near which the males were released. The implication here is that a primary requirement for successful application of a sterile male technique is to locate and make releases at individual spot infestations. The shipboard experiment during Phase II indicated this could be accomplished with a thorough trapping program Peaks of highest density indicate the location of spot infestations. Also, in B. germanica, the number of sterile males to be released should be predicated on numbers of females expected to mature within a given time period, not on total population density. This applies because our research indicated that the species is predominantly monogamous.

It is generally difficult to study free populations because most situations are subject to disturbance by people. The experiments on an inactive ship were therefore of unusual interest. They provided the first measure of the growth rate of a free population under essentially optimal conditions. Much was learned concerning the use and misuse of trapping data - an important aspect because this is the tool most frequently used to assess the impact of control measures. Another facet of importance to control was the evidence that spot infestations, once established, remain inhabited, even if in comparatively unfavorable locations. Furthermore, new spot infestations were established due to

insecticide-induced dispersal. After each treatment, we found the shipboard population to be more widely dispersed than prior to treatment. Small spot infestations in new areas can serve as feeders of cockroaches moving back to more favored locations. Treatments where containment is achieved will be far more successful in preventing population regrowth than those where cockroaches escape into untreated refugia. Lastly, a first step was taken towards addressing one of the major problems that face pest control operators - whether and to what degree a particular level of resistance will affect the efficacy of an insecticide treatment.

A behavioral difference between a resistant field strain and a susceptible laboratory strain was discovered in the course of Ph.D. research by B. L. Bret. The significance of this finding to control strategies cannot be assessed. No further information is available on behavioral polymorphism in cockroach populations, much less whether they are likely to affect control efforts. This is an area that certainly deserves attention in future research.

As a result of the pheromone and other studies, it is to be hoped that attention will be given to differences with age/sex class and female reproductive state in investigations on cockroach behavior. The pheromone studies will be valuable from the point-of-view of control if eventually pheromones are used in control programs. For example, the dispersant may have potential for repelling

cockroaches from cracks and crevices where they might find refuge.

In conclusion, we note that the results of ONR-supported research have been reported at many scientific meetings, including the Eastern Branch of the Entomological Society of America, national meetings of the same society, a recent National Conference on Urban Entomology, and the 1980 International Congress of Entomology.

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